Immunology and Cell Biology (2001) 79, 132–140

(10) Asthma

EXHIBIT
10
10/827,294

Special Feature

Tumour necrosis factor-α: The role of this multifunctional cytokine in asthma

PAUL S THOMAS

Faculty of Medicine, University of New South Wales and Department of Respiratory Medicine, Prince of Wales Hospital, Randwick, New South Wales, Australia

Summary Tumour necrosis factor-α (TNF-α) is recognized as an important mediator in many cytokine-dependent inflammatory events. It is known that TNF-α is released in allergic responses from both mast cells and macrophages via IgE-dependent mechanisms, and elevated levels have been demonstrated in the bronchoalveolar fluid (BALF) of asthmatic subjects undergoing allergen challenge. Inhaled TNF-α increases airway responsiveness to methacholine in normal and asthmatic subjects associated with a sputum neutrophilia. Additional data indicate that TNF-α can upregulate adhesion molecules, facilitate the immigration of inflammatory cells into the airway wall and activate pro-fibrotic mechanisms in the subepithelium. These data suggest that TNF-α plays a role in the initiation of allergic asthmatic airway inflammation and the generation of airway hyper-reactivity. In addition, polymorphisms of the TNF-α gene 5′ untranslated region, particularly at –308 bp, have been described as being associated with asthma. This polymorphism is associated with increased levels of TNF-α, but as yet, no asthma studies have demonstrated a phenotypic difference between those individuals with the polymorphism and those with the wild type gene. The TNF receptors (TNF-R p55 and p75), also known as CD120a and b, have also been shown to be present in the lung, but their functional importance is only just emerging. In asthma, TNF may function as a pro-inflammatory cytokine that causes the recruitment of neutrophils and eosinophils. Treatment directed specifically at a reduction in TNF-α activity may conceivably be useful as a glucocorticosteroid-sparing asthma therapy.

Key words: airway inflammation, airway-reactivity, asthma, mast cells, tumour necrosis factor.

Introduction

In the last decade it has become evident that cytokines play a pivotal role in the pathogenesis of asthma. The role of antigen-induced tumour necrosis factor-α (TNF-α) release and antigen stimulation of other cytokines is an important area of study. In addition to TNF-α, cytokines including interleukin (IL)-1β, IL-2, IL-3, IL-4, IL-5, IL-8, granulocyte-macrophage-colony stimulating factor (GM-CSF), and interferon-γ (IFN-γ) have also been implicated in the development of the asthmatic inflammatory response. This review will only attempt to cover the area relating to TNF-α, but multiple cytokine networks make interpretation of *in vitro* experimental data quite difficult. If selective inhibition of a given cytokine or mediator is possible, and this inhibition results in a favourable response in clinical asthma, then the importance of that cytokine is firmly established.

Tumour necrosis factor-α is a cytokine usually associated with cell-mediated immunological responses that have been classified on the basis of studies in mice. It is becoming apparent that inbred murine models of immunology may be limited by species differences, and do not reflect the true situation as seen in those diseases experienced by man. Asthma is perceived as a T-helper type 2 (Th2) disease with a particular profile of cytokine release, which is thought to include IL-4 and IL-5. Increasing evidence indicates that other cytokines, which in mice are classically considered to belong to Th1-type profiles, are also associated with the inflammatory response that characterizes human asthma. One such mediator is TNF-α, which has been implicated in asthmatic inflammation by a broad series of subcellular, *in vitro*, ex-vivo, *in vivo* and genetic studies (Fig. 1).

Correspondence: Dr PS Thomas, Department of Respiratory Medicine, Prince of Wales Hospital, Randwick, NSW 2031, Australia. Email: paul.thomas@unsw.edu.au

Received 15 September 2000; accepted 15 September 2000.

Cellular origin of TNF-α in asthmatic responses

Tumour necrosis factor-α production was first described in macrophages and monocytes.[1] Since then, other cells of the haemopoietic lineage have been shown to have an ability to generate this cytokine de novo. The sensitized mast cell is known to store a number of cytokines, including TNF-α, within its granules and to release them upon antigenic presentation, making it a pivotal cell in the allergic asthmatic response to allergen.[2,3] In addition, other cells in the airway have the ability to generate TNF-α; eosinophils,[4,5] epithelial cells[6] and airway macrophages.[7]

The acute asthmatic response to allergen is mediated by sensitized mast cells whose high-affinity IgE receptors (FcεRI receptors) are occupied by IgE directed against specific allergens, but the late or delayed response occurring hours later is mediated by a number of different cells. Allergen cross-links IgE molecules attached to mast cell surface FcεRI receptors, causing degranulation. Mast cells have been shown to generate a range of mediators including cytokines, and recent

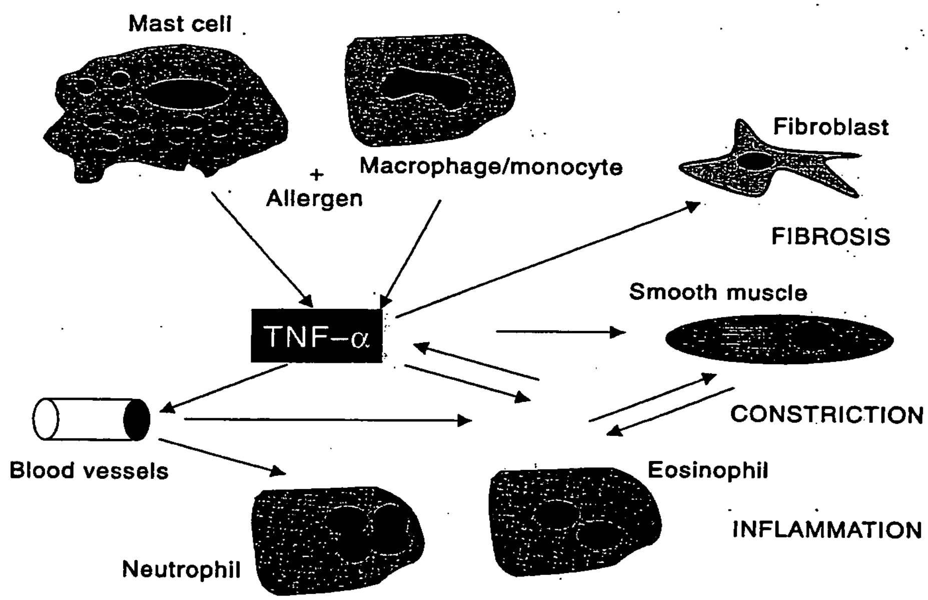

Figure 1 Schematic diagram of the ways in which TNF-α may interact with other cells within the airway.

work has documented that the mast cell granule itself contains preformed TNF-α.[3,8] This indicates that this cytokine will be coreleased with the more extensively characterized preformed mast cell granule mediators, such as histamine, chymase and tryptase. Mast cell mediators are classically associated with immediate bronchospasm, and now TNF-α has also been shown to induce airway hyper-reactivity.[9,10] *In vitro*, this smooth muscle hyper-responsiveness appears to be immediate, but *in vivo* the effects are detected later. The mechanisms behind this characteristic hyper-responsiveness, which is associated with asthma, are being clarified in part by an understanding of the pro-inflammatory cell influx.

Tumour necrosis factor-α, adhesion molecules and airway inflammatory cell recruitment

Tumour necrosis factor-α is a chemotactic cytokine for granulocytes including eosinophils and neutrophils,[11] probably by up-regulating cellular adhesion molecules. Tumour necrosis factor-α is known to up-regulate adhesion molecules, such as E-selectin, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1 or CD54);[12,13] and the latter molecule has been implicated in a simian model of asthma.[14] Tumour necrosis factor-α induction of adhesion molecules, such as VCAM-1, on pulmonary endothelium is important for eosinophil recruitment[15,16] and in addition, biopsies of asthmatic bronchial wall have shown VCAM-1 to be up-regulated.[17]

Both TNF-α and IL-1β increase the expression of ICAM-1 and VCAM-1 on respiratory epithelial cells *in vitro*, and eosinophils show increased adherence to these cells after stimulation, although blocking experiments suggest that CD11/CD18 (β2) integrins may play an important role in this adhesion.[18,19] These effects are amplified in the presence of IL-5, perhaps via a CD18-dependent mechanism.[20] The TNF-α-induced increase in ICAM-1 also aids *in vitro* binding of activated T lymphocytes to airway smooth muscle cells, which is inhibited by cyclic AMP-dependent protein kinases.[21] Thus, TNF-α is associated with the up-regulation of adhesion molecules, and is able to facilitate inflammatory cell migration.

Effects of TNF-α at the cellular level

Tumour necrosis factor-α has been demonstrated to cause an increase in airway hyper-reactivity.[10] This increased airway smooth muscle responsiveness may be via the recruitment of inflammatory cells,[22] by direct effects upon airway smooth muscle,[23] or by generating a cascade of inflammatory responses with the release of mediators, including increased sensitization with elevated histamine release.[24]

Tumour necrosis factor-α causes changes in the ionized calcium flux within smooth muscle, and also increases mitogenic activity via the TNF p55 receptor.[25–27] Of particular interest is that it can also cause an increase in smooth muscle eotaxin generation and secretion (along with IL-1β) from human airway smooth muscle, with eotaxin being clearly demonstrated within asthmatic airway muscle.[28] In addition, increased TNF-α in the bronchoalveolar fluid of ovalbumin-sensitized guinea pigs appeared to stimulate airway smooth muscle cells to secrete endothelin-1 (ET-1) and thus induce GM-CSF mRNA expression in fibroblasts,[29] suggesting an indirect, novel mechanism for induction of airway wall fibrosis. This increases the complexity of the known mechanisms of airway inflammation, as it indicates that once activated, the smooth muscle itself can induce further cytokine-mediated inflammation, including events associated with subepithelial fibrosis.

The myofibroblast is a cell that has become of interest in asthma research in recent years. Increased numbers of these cells have been identified in the subepithelium of asthmatic subjects.[30] Tumour necrosis factor-α is implicated in

myofibroblast proliferation in response to cytokines,[31] as well as its classical activity upon fibroblasts by increasing mitogenicity and receptivity to other mitogens.[32,33] Myofibroblasts lie below the bronchial basement membrane, ideally situated to influence airway wall fibrosis and inflammatory cells, and TNF-α may therefore contribute to airway wall fibrosis by stimulating these cells. In response to myofibroblast-conditioned media, eosinophils ex-vivo show increased survival and less apoptosis, probably via GM-CSF acting as a mediator.[34] Tumour necrosis factor-α may have additional indirect remodelling activity because it is able to induce eosinophils to release the matrix metalloproteinase, MMP-9,[35] and to stimulate glycosoaminoglycan synthesis in human lung fibroblasts.[36] Thus, there are direct lines of evidence suggesting that soluble TNF-α activates myofibroblasts and fibroblasts, leading to the generation of subepithelial airway fibrosis in asthma. Additional direct effects of TNF-α upon the airway mucosa have been noted, such as the stimulation of mucus secretion,[37] one of the markers of airway inflammation.

Regulation of cytokines appears to occur in levels of increasing complexity, indicating the necessity of interpreting *in vitro* data in the light of clinical information from asthmatic subjects. Alveolar macrophages from atopic asthmatic subjects enhanced IL-5 production from $CD4^+$ cells and this was reduced by anti-TNF-α antibodies (as also occurred with neutralizing antibodies to IL-1α, IL-1β and IL-6).[38] These data confirm earlier work in the mouse that reports that mice treated with anti-TNF antibodies have reduced levels of IL-5 expression.[39] Also, cross-regulation of TNF-α by IL-4 and IL-5 has been demonstrated *in vitro* and *in vivo* with down-regulation of TNF-α by IL-4.[40] In addition to up-regulation of adhesion molecules, and the induction of IL-5, TNF-α is able to synergise with other cytokines to promote activation of eosinophils.[41] The IL-4 repression of TNF-α production might be a method of negative feedback control. Nonetheless, eosinophils themselves are a source of TNF-α and this is increased in allergic inflammation.

These data indicate the potential variety of roles that TNF-α can play in asthma by increasing smooth muscle responsiveness, activating myofibroblasts and fibroblasts, and by regulating the activity of eosinophils via IL-4 and IL-5.

Tumour necrosis factor-α in experimental and clinical asthma

The data that are emerging in experimental and clinical asthma studies are suggesting that TNF-α plays a role in human asthma. Mast cell granules have been shown to contain TNF-α by electron immunocytochemistry and by immunoblot, which is localized to the mast cell granule.[3] This mediator is released by passive sensitization and challenge with allergen, both *in vitro*[3] and *in vivo*.[42,43] Thus, mast cell-associated TNF-α was predicted to be released in allergic asthmatic subjects upon allergen challenge. These predictions have been borne out by the following observations in animal models of asthma and in the human clinical disease.

While the presence of the eosinophil is recognized as the hallmark of asthmatic inflammation, evidence for TNF-α-mediated neutrophil involvement in the pathogenesis of asthma is increasing and is supported by the fact that neutrophils from asthmatic subjects show increased migratory responses,[44,45] increased superoxide generation, and that their secretory products increase bronchial ring contractility.[46–48] Airway neutrophilia is associated with some subtypes of asthma,[49–51] and of course, with bacterial inflammation causing exacerbations of asthma. Neutrophils release preformed mediators, such as elastase, and also an array of newly formed mediators, such as superoxide radicals, leukotrienes and prostaglandins, many of which cause an increase in bronchial reactivity. Tumour necrosis factor-α released by macrophages in response to bacteria will maintain and amplify the neutrophil response because they appear to release TNF-α over a longer time period than the mast cell.

Animal studies have clearly shown that bronchial hyper-responsiveness can be induced in rats exposed to endotoxin, which is known to increase the generation of TNF-α from bronchial and alveolar macrophages, and TNF-α levels were elevated in the broncho-alveolar lavage (BAL) from these rats. This increase in murine bronchial hyper-responsiveness was found to be mimicked by the administration of recombinant TNF-α[52,53] and to be significantly abrogated by the administration of anti-TNF-α antibodies. Neutrophilia was seen in the BAL from rats after TNF-α administration, implicating this cell in the generation of endotoxin hyper-responsiveness,[9] and mimicking the increased responsiveness seen after respiratory tract infections. Administration of endotoxin to asthmatic subjects increases bronchial reactivity, with TNF-α as the probable mediator.[54] The increase in TNF-α levels seen with increased bronchial reactivity after inhalation of bacterial lipopolysaccharide (LPS), would mimic the asthmatic response in bacterial infections.[54] Asthmatic subjects whose peripheral blood monocytes were stimulated by LPS, showed an increase in production of TNF-α, IL-8 and GM-CSF compared to normal subjects;[55] and similar results were found when asthmatic BAL leucocytes were stimulated with PMA and PHA.[56]

Human studies on asthmatic subjects reveal an increase in the generation of TNF-α in macrophages and peripheral blood monocytes after antigen challenge[7,57], and increased TNF mRNA in asthmatic airway lavage.[58] In a study of eight normal subjects who inhaled nebulized TNF-α, there was a significant rise in sputum neutrophils and a significant increase in methacholine responsiveness, which is a measure of airway reactivity.[10] A single dose of 60 ng recombinant human TNF-α caused a leftward shift in the methacholine concentration response curves and a fall in the methacholine log provocative concentration (PC), which caused a 15% fall in forced expiratory volume in 1 s (PC_{15}, FEV_1) at all time points compared with control, reaching a maximum at 24 h and persisting up to 48 h. There was no change in spirometry. There was also a significant neutrophil influx seen in the induced sputum, again reaching a maximum at 24 h. These findings have recently been replicated in mild asthmatic subjects (Thomas *et al.*, unpubl. data, 1999).

These clinical studies would appear to be confirmed by clinical studies of allergic asthma. Resting alveolar macrophages and peripheral blood monocytes in asthmatic subjects secrete increased levels of TNF-α,[7] and after allergen challenge there is a further increase in TNF-α supernatant levels from these cells at the time of the late asthmatic response.[57] Passive sensitization of these cells and exposure to anti-IgE

to cross-link the FcεRII receptors on monocytes led to increased TNF-α (and IL-6) production, while additional IFN-γ had a synergistic effect on the stimulation by anti-IgE. Increased release of TNF-α and IL-1β was seen from peripheral blood monocytes ex-vivo in di-isocyanate-sensitive asthma, and other cases of occupational asthma,[59,60] and was also demonstrated *in vivo*[61] although no changes were seen in bronchial biopsies for ICAM-1 and E-selectin.

Increased sputum TNF-α and IL-5 levels were detected in allergic asthmatic subjects 24 h after allergen challenge,[62] and also in the serum of atopic subjects in association with IL-1β.[63] Tumour necrosis factor-α was also reported to be increased during asthma exacerbations,[64] and in this situation to be associated with increased VCAM-1 and serum-soluble ICAM-1 and E-selectin.[65] These findings have been confirmed independently by noting increased TNF-α (and IL-6) in acute asthma,[66,67] as well as other studies which have shown a correlation with eosinophil cationic protein.[68]

More recently, Nocker *et al.*[69] found TNF-α increased above baseline in a segmental allergen challenge model, but in both asthmatic subjects and controls, and in a separate study, TNF-α mRNA was found to be ubiquitously detected in induced sputum from both normal and asthmatic subjects.[70] Similarly, increased TNF-α secretion was detected from bronchial lavage T lymphocytes ex-vivo at baseline, along with IL-13, IFN-γ and GM-CSF, IL-3, IL-4 and IL-5 at 24 h.[71] Not all studies have confirmed these results, including a study of a large number of asthmatic subjects, where TNF-α immunostaining and mRNA was less common in the bronchial biopsies and lavage of untreated asthmatic subjects, compared to those treated with glucocorticosteroid therapy.[72] The reason for this difference is not clear, but perhaps IL-4 or another mediator, such as nitric oxide, could be switching off TNF-α production.

An increase in exhaled nitric oxide is strongly associated with asthmatic inflammation, and TNF-α stimulation is in turn associated with increased inducible nitric oxide synthase (iNOS) in bronchial epithelial cells, which is usually observed when stimulation occurs in the presence of other cytokines.[73,74] Tumour necrosis factor-α may therefore be linked to the induction of iNOS seen in asthma,[75] which in turn increases exhaled levels of nitric oxide.[76]

Thus, increasing evidence indicates that TNF-α is responsible for the smooth muscle activation and late phase inflammatory responses seen in asthma, which are associated with inflammatory cell influx. This concurs with the current concept of the late reaction in asthma being mediated by an inflammatory response, as seen by the influx of inflammatory cells, which include eosinophils, neutrophils and lymphocytes. Tumour necrosis factor-α also appears to play a key role in other inflammatory diseases, such as rheumatoid arthritis, as judged by the favourable response of this disease to anti-TNF-α monoclonal antibodies in man.[77] Mast cell-derived TNF-α release is associated with the responses in allergic asthma, but cells other than the mast cell are able to generate TNF-α, including the pulmonary macrophage, which may well play such a role within the lung, since it possesses the FcεRII and III receptors, and is activated by specific antigen presentation. Unlike the mast cell, the pulmonary macrophage has no preformed TNF-α, but is a potent source of the newly generated TNF-α that is released for several hours after stimulation. This would also be an important source of TNF-α in bacterial infection.

From the majority of these studies, in both animal models and in human asthma, TNF-α appears to be implicated in the allergic asthmatic response.

Tumour necrosis factor receptors

Tumour necrosis factor-α acts via two related receptors, originally distinguished by molecular size fractionation. They are designated p55 and p75, or CD120a (TNF-R1) and CD120b (TNF-R2), respectively. Other members of the TNF receptor superfamily have been characterized, and some have a 'death domain' in the intracellular region of the transmembrane receptor that can couple and activate caspases.[78–80] These include TNF-R1/CD120a, Fas/apoptosis-inducing receptor (APO)-1, death receptor (DR)3, DR6, TNF-related apoptosis-inducing ligand (TRAIL)-R1 and TRAIL-R2. In certain situations, autotropic TNF-R1 activation can occur, while TNF-R2 can also contribute to the TNF-R1 cytotoxicity. As indicated above, TNF-α activation of cells can generate responses other than apoptosis and cell death. Other downstream effects of TNF receptor activation include the upregulation of MMP-9, which can be via an autocrine TNF-R mechanism.[81] Soluble forms of many of these cell surface receptors are found and may act to bind and neutralize circulating TNF-α. Expression of the TNF-R1 and R2 receptors has now been described on airway monocytes, macrophages, lymphocytes and granulocytes in bronchoalveolar lavage,[82] while the 4–1BB receptor, lymphotoxin beta-R and Fas have also been described within the lung.[83] Circadian rhythms of soluble TNF-R2 have been described with a peak at 8 AM, which may have relevance for asthma.[84] As yet, there are few data to indicate the importance of the TNF receptor family in allergic asthma.

Genotype studies

If the induction of increased levels of local TNF-α is associated with asthma, then it is a logical step to consider genotypic analysis of families and communities with asthma, to see if asthma is more common in those who have the ability to generate higher levels of TNF-α, by virtue of polymorphic variants. One such candidate is the −308 polymorphism. The −308 TNF-α promoter polymorphism is a bi-allelic G (TNF1 allele) to A (TNF2 allele) polymorphism 308 nucleotides upstream of the transcription initiation site. The TNF2 genotype is associated with elevated plasma TNF-α levels, and also with higher amounts of TNF on stimulation *in vivo* and ex-vivo.[85] There is also an association of the TNF2 genotype with the MHC haplotype HLA A1, B8, DR3,[86] and with adverse outcomes secondary to cerebral malaria and other diseases, where there were exceptionally elevated TNF-α levels.[87]

A number of independent reports have indicated an association of this polymorphism with asthma.[88–90] Albuquerque *et al.* demonstrated an association of the TNF-α −308 polymorphism with a five-fold risk of diagnosed asthma, as was the LT alpha Nco I locus,[91] while others have shown an association between the −308 TNF-α promoter polymorphism and bronchial reactivity (but not the lymphotoxin alpha Nco I locus).[92] Likewise, Changani *et al.*[93] indicated that the −308

polymorphism was associated with asthma, but not specifically with those with fatal/near fatal asthma. The diverse geographical nature of these reports suggests that there is relevance in these findings, but not all studies have found an association. Some of these latter reports are published in abstract form only, presumably because it is more difficult to publish a lack of an association of a polymorphism with a disease.[94]

Kroeger *et al.*[95] demonstrated *in vitro* that the −308 polymorphism has cell and stimulus specificity when tested in transformed cell lines (only U937 and Jurkat cells, and not in cells from a B cell line, HeLa cells, HepG2 cells or a monocyte cell line, THP-1). Using footprint analysis these findings have been extended to suggest that there is a hypersensitive site at −308. These experiments did not show a difference in the affinity for DNA binding proteins, but indicated that TNF2 polymorphism was a much stronger transcriptional activator than TNF1 in a B cell line.[96] Among others, Uglialoro *et al.*[97] described three separate TNF-α polymorphisms, however, functional importance has yet to be established for these or other polymorphisms.

If a cytokine such as TNF-α has been shown to generate airway inflammation and increased airway responsiveness, it would be surprising if there was no association between the propensity to generate increased levels of TNF-α and either asthma or increased bronchial reactivity. It remains to be seen whether this is a subset or part contributor to the overall genotype that predisposes to allergic asthma.

Tumour necrosis factor-α release

Tumour necrosis factor-α is synthesized as a 26 kDa membrane-bound protein. The TNF-α ectodomain is cleaved at the cell surface to a soluble 17 kDa protein by a metalloproteinase-like enzyme that has been designated TNF-α converting enzyme (TACE). The 17 kDa protein is considered the mature product, but there are data to suggest that the 26 kDa membrane-associated protein could be implicated in direct cell:cell interactions. The tumour necrosis factor-α converting enzyme is also known as ADAM 17 as it is part of a larger ADAM family (ADAM: proteins containing a disintegrin and metalloproteinase domain), and is inhibited by tissue inhibitor of metalloproteinase-3 (TIMP-3).[98] This enzyme is also responsible for the liberation of other membrane-bound proteins, including TNF receptors, transforming growth factor-α, and the adhesion molecule, L-selectin.[99,100] Various studies have indicated that is possible to inhibit TACE, and hence TNF-α release, by agents such as, hydroxamic acid derivatives.[2,101] These inhibitors may have less specificity upon TNF-α than was first thought, perhaps because TACE has other functions than just cleaving TNF-α. As yet, there are few reports relating to MMP in asthma, and none on TACE or TIMP3 in this condition.

Therapeutic potential

Glucocorticosteroid (GCS) treatment of asthma is the most effective anti-inflammatory agent and has a broad range of activity across many cytokine networks and other mediators.[102] Inhibition of TNF-α production is no exception to this activity. The breadth of this inhibition and activity also leads to unwanted side-effects at higher doses, and when the treatment period is prolonged. There is therefore a need for increasing the range of GCS-sparing treatments that can be used to reduce the dose of these highly effective drugs. Novel methods of inhibiting TNF-α are currently under investigation in diseases other than asthma, for example, rheumatoid arthritis and conditions where an excess of TNF-α contributes to morbidity and mortality (e.g. malaria and Gram-negative sepsis, and the Jarish Herxheimer reaction). A variety of candidates are being studied. These are postulated to have different mechanisms, including inhibitors of TNF-α mRNA transcription (e.g. pentoxifylline and phosphodiesterase inhibitors).[101,103,104] Entzian *et al.*[104] studied three xanthines and showed both inhibition of IFN-α and TNF-α release with the novel compound A802715 demonstrating greater potency than pentoxifylline or theophylline. Other types of pharmacological TNF-α inhibitors include accelerators of TNF-α mRNA degradation (e.g. thalidomide);[105–107] inhibitors of TNF protein translation (e.g. tetravalent guanylhydrazones);[108] and the metalloproteinase inhibitors that prevent the cleavage of the 26 kDa membrane-bound protein to the active 17 kDa molecule.[101,109,110] Other approaches include TNF receptor fusion proteins[111] and monoclonal antibodies. Monoclonal antibodies have also been raised against TNF-α and have reached trials in human subjects who have rheumatoid arthritis, usually as a humanized murine antibody.[77,112] Generally, these studies have shown encouraging results, although the problems associated with this type of therapy may limit its use to certain categories of disease.

Summary

Tumour necrosis factor-α increases the expression of cellular adhesion molecules and facilitates the passage of leucocytes into the airway in response to allergen and to bacterial products. In addition, it would appear to increase airway smooth muscle cell contractility and expression of eotaxin, and also to increase IL-5 secretion. In asthma, as in other situations, TNF-α may have apoptotic activity, although this specific question has not been addressed within the airway, but perhaps it could be responsible for airway epithelial shedding. There are also data to implicate TNF-α in airway remodelling and fibrosis. A polymorphism in the TNF-α promoter resulting in increased generation of this cytokine has also been linked to asthma in genotypic studies. These facts make TNF-α a logical target for intervention and studies are underway to determine if inhibition of this multifunctional cytokine may improve the range of drugs available in asthma therapy.

Acknowledgements

This review was funded in part by the National Health & Medical Research Council (Australia), Asthma New South Wales, the Viertel and the Ramaciotti Foundations. The author wishes to thank his collaborators including Dr Deborah H. Yates, and those at the Inflammation Research Unit, UNSW (particularly Gavin Heywood, Colleen Bruce, Drs John Hunt and Nick DiGirolamo and Associate Professors Rakesh Kumar and Patrick McNeil).

References

1 Pennica D, Nedwin GE, Hayflick JS *et al.* Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. *Nature* 1984; **312**: 724–9.

2 Plaut M, Pierce JH, Watson CJ, Hanley-Hyde J, Nordan RP, Paul WE. Mast cell lines produce lymphokines in response to cross-linkage of FcεRI or to calcium ionophores. *Nature* 1989; **339**: 64–7.

3 Thomas PS, Pennington DW, Schreck RE, Levine TM, Lazarus SC. Authentic 17kD tumor necrosis factor α is synthesised and released by canine mast cells and up-regulated by stem cell factor. *J. Clin. Exp. Allergy* 1996; **26**; 710–18.

4 Costa JJ, Matossian K, Resnick MB *et al.* Human eosinophils can express the cytokines tumor necrosis factor alpha and macrophage inflammatory protein-1 alpha. *J. Clin. Invest.* 1993; **91**: 2673–84.

5 Finotto S, Ohno I, Marshall JS *et al.* TNF alpha production by eosinophils in upper airways inflammation (nasal polyposis). *J. Immunol.* 1994; **153**: 2278–89.

6 Khair OA, Devalia JL, Abdelaziz MM, Sapsford RJ, Tarraf H, Davies RJ. Effect of Haemophilus influenzae endotoxin on the synthesis of IL-6, IL-8, TNF alpha and expression of ICAM-1 in cultured human bronchial epithelial cells. *Eur. Respir. J.* 1994; **7**: 2109–16.

7 Gosset P, Tsicopoulos A, Wallaert B *et al.* Increased secretion of tumor necrosis factor α and interleukin-6 by alveolar macrophages consecutive to the development of the late asthmatic reaction. *J. Allergy Clin. Immunol.* 1991; **88**: 561–71.

8 Thomas PS, Schreck RE, Ruoss SJ, Lazarus SC. Heterogeneity of intact mast cell granules purified from canine mastocytoma cell lines. *Am. J. Physiol.* 1991; **4**: L153–60.

9 Kips J, Tavernier J, Pauwels R. Tumor necrosis factor causes bronchial hyperresponsiveness in rats. *Am. Rev. Respir. Dis.* 1992; **145**: 332–6.

10 Thomas PS, Yates DH, Barnes PJ. Tumor necrosis factor alpha increases airway responsiveness and sputum neutrophilia in normal subjects. *Am. J. Respir. Crit. Care Med.* 1995; **152**: 76–80.

11 Ming WJ, Bersani L, Mantovani A. Tumor necrosis factor is chemotactic for monocytes and polymorphonuclear leukocytes. *J. Immunol.* 1987; **87**: 1469–74.

12 Gamble JR, Harlan JM, Klebanoff SJ, Vadas MA. Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumor necrosis factor. *Proc. Natl Acad. Sci. USA* 1985; **82**: 8667–71.

13 Pober JS, Gimbrone MA Jr *et al.* Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor, and immune interferon. *J. Immunol.* 1986; **137**: 1893–6.

14 Wegner CD, Gundel RH, Reilly P, Haynes N, Letts G, Rothlein R. Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. *Science* 1990; **247**: 456–9.

15 Lassalle P, Gosset P, Delneste Y *et al.* Modulation of adhesion molecule expression on endothelial cells during the late asthmatic reaction: role of macrophage-derived tumor necrosis factor-alpha. *Clin. Exp. Immunol.* 1993; **94**: 105–10.

16 Yamamoto H, Sedgwick JB, Busse WW. Differential regulation of eosinophil adhesion and transmigration by pulmonary microvascular endothelial cells. *J. Immunol.* 1998; **161**: 971–7.

17 Fukada T, Fukshima Y, Numao T, Ando N, Arima M, Nakajima H *et al.* Role of interleukin-4 and vascular cell adhesion molecule-1 in selective eosinophil migration into the airways in allergic asthma. *Am. J. Respir. Cell Mol. Biol.* 1996; **14**: 84–94.

18 Godding V, Stark JM, Sedgwick JB, Busse WW. Adhesion of activated eosinophils is enhanced by tumor necrosis factor alpha and interleukin-1 beta. *Am. J. Respir. Crit. Care Med.* 1995; **13**: 555–62.

19 Moser R, Fehr J, Olgiati L, Bruijnzeel PL. Migration of primed human eosinophils across cytokine-activated endothelial cell monolayers. *Blood* 1992; **79**: 2937–45.

20 Ebisawa M, Liu MC, Yamada T *et al.* Eosinophil transendothelial migration induced by cytokines. II. Potentiation of eosinophil transendothelial migration by eosinophil-active cytokines. *J. Immunol.* 1994; **152**: 4590–6.

21 Panettieri RA Jr, Lazaar AL, Pure E, Albelda SM. Activation of cAM-dependent pathways in human airway smooth muscle cells inhibits TNF alpha-induced ICAM-1 and VCAM-1 expression and T lymphocyte adhesion. *J. Immunol.* 1995; **154**: 2358–65.

22 Shah A, Church MK, Holgate ST. Tumour necrosis factor alpha: a potential mediator of asthma. *Clin. Exp. Allergy* 1995; **25**: 1038–44.

23 Anticevich SZ, Hughes JM, Black JL, Armour CL. Induction of human airway responsiveness by tumour necrosis factor alpha. *Eur. J. Pharmacol.* 1995; **284**: 221–5.

24 Hughes JM, Stringer RS, Black JL, Armour CL. The effects of tumour necrosis factor alpha on mediator release from human lung. *Pulm. Pharmacol.* 1995; **8**: 31–6.

25 Amrani Y, Panettieri RA Jr, Frossard N, Bronner C. Activation of the TNF alpha-p55 receptor induces myocyte proliferation and modulates agonist-evoked calcium transients in cultured human tracheal smooth muscle cells. *Am. J. Respir. Cell Mol. Biol.* 1996; **15**: 55–63.

26 Amrani Y, Krymskaya V, Maki C, Panettieri RA Jr. Mechanisms underlying TNF-alpha effects on agonist-mediated calcium homeostasis in human airway smooth muscle cells. *Am. J. Physiol.* 1997; **273**: L1020–8.

27 Parris JR, Cobban HJ, Littlejohn AF, MacEwan DJ, Nixon GF. Tumour necrosis factor alpha activates a calcium sensitization pathway in guinea-pig bronchial smooth muscle. *J. Physiol.* 1999; **518**: 561–9.

28 Ghaffar O, Hamid Q, Renzi PM *et al.* Constitutive and cytokine-stimulated expression of eotaxin by human airway smooth muscle cells. *Am. J. Respir. Crit. Care Med.* 1999; **159**: 1933–42.

29 Xu J, Zhong NS. The interaction of tumour necrosis factor alpha and endothelin-1 in pathogenetic models of asthma. *Clin. Exp. Allergy* 1997; **27**: 568–73.

30 Brewster CE, Howarth PH, Djukanovic R, Wilson J, Holgate ST, Roche WR. Myofibroblasts and subepithelial fibrosis in bronchial asthma. *Am. J. Respir. Cell Mol. Biol.* 1990; **5**: 507–11.

31 Rubbia-Brandt L, Sappino AP, Gabbiani G. Locally applied GM-CSF induces the accumulation of alpha-smooth muscle actin containing myofibroblasts. *Virchows Arch. B (Cell Path)* 1991; **60**: 73–82.

32 Paulsson Y, Austgulen R, Hofsli E, Heldin CH, Westermark B, Nissen-Meyer J. Tumor necrosis factor-induced expression of platelet-derived growth factor A-chain messenger RNA in fibroblasts. *Exp. Cell Res.* 1989; **180**: 490–6.

33 Palombella VJ, Mendelsohn J, Vilcek J. Mitogenic action of tumor necrosis factor in human fibroblasts: interaction with epidermal growth factor and platelet-derived growth factor. *J. Cell. Physiol.* 1988; **135**: 23–31.

34 Zhang S, Mohammed Q, Burbidge A, Morland CM, Roche WR. Cell cultures from bronchial subepithelial myofibroblasts enhance eosinophil survival in vitro. *Eur Respir J.* 1996; **9**: 1839–46.

35 Schwingshackl A, Duszyk M, Brown N, Moqbel R. Human eosinophils release matrix metalloproteinase –9 on stimulation with TNF alpha. *J. Allergy Clin. Immunol.* 1999; **104**: 983–9.

36 Elias JA, Krol RC, Fruendich B, Sampson PM. Regulation of human lung fibroblast glycosoaminoglycan production by recombinant interferons, tumor necrosis factor, and lymphotoxin. *J. Clin. Invest.* 1988; **81**: 325–33.

37 Levine SJ, Larivee P, Logun C, Angus CW, Ognibene FP, Shelhamer JH. Tumour necrosis factor alpha induces mucin hypersecretion and MUC-2 gene expression by human airway epithelial cells. *Am. J. Respir. Cell Mol. Biol.* 1995; **12**: 196–204.

38 Tang C, Rolland JM, Li X, Ward C, Bish R, Walters EH. Alveolar macrophages from atopic asthmatics, but not atopic non-asthmatics, enhance interleukin-5 production by CD4+ T cells. *Am. J. Respir. Crit. Care Med.* 1998; **157**: 1120–6.

39 Zhang K, Gharaee-Kermani M, McGarry B, Remick D, Phan SH. TNF-alpha-mediated lung cytokine networking and eosinophil recruitment in pulmonary fibrosis. *Immunology* 1997; **158**: 954–9.

40 Hart PH, Vitti GF, Burgess DR, Whitty GA, Piccoli DS, Hamilton JA. Potential anti-inflammatory effects of interleukin 4: suppression of human monocyte tumor necrosis factor alpha, interleukin 1, and prostaglandin E2. *Proc. Natl Acad. Sci. USA* 1989; **86**: 3803–7.

41 Luttman W, Matthiesen T, Matthys H, Virchow JC Jr. Synergistic effects of interleukin-4 or interleukin-13 and tumour necrosis factor alpha on eosinophil activation in vitro. *Am. J. Respir. Crit. Care Med.* 1999; **20**: 474–80.

42 Walsh LJ, Trinchieri G, Waldorf HA, Whitaker D, Murphy GF. Human dermal mast cells contain and release tumor necrosis factor α, which induces endothelial leukocyte adhesion molecule 1. *Proc. Natl Acad. Sci. USA* 1991; **88**: 4220–4.

43 Wershill BK, Zhen-Sheng W, Gordon JR, Galli SJ. Recruitment of neutrophils during IgE-dependent cutaneous late phase reactions in the mouse is mast cell-dependent. *J. Clin. Invest.* 1991; **87**: 446–53.

44 Klebanoff SJ, Vadas MA, Harlan JM *et al.* Stimulation of neutrophils by tumor necrosis factor. *J. Immunol.* 1986; **136**: 4220–5.

45 Nagy L, Lee TH, Kay AB. Neutrophil chemotactic activity in antigen-induced late asthmatic reactions. *N. Eng. J. Med.* 1982; **306**: 497–501.

46 Håkansonn L, Carlson M, Stålenheim G, Venge P. Migratory responses of eosinophil and neutrophil granulocytes from patients with asthma. *J. Allergy Clin. Immunol.* 1990; **85**: 743–50.

47 Meltzer S, Goldberg B, Lad P, Easton J. Superoxide generation and its modulation by adenosine in the neutrophils of subjects with asthma. *J. Allergy Clin. Immunol.* 1989; **83**: 960–6.

48 Hallahan AR, Armour CL, Black JL. Products of neutrophils and eosinophils increase the responsiveness of human isolated bronchial tissue. *Eur. Respir. J.* 1990; **3**: 554–8.

49 Boschetto P, Mapp CE, Picotti G, Fabbri LM. Neutrophils and asthma. *Eur Respir J. (Suppl.)* 1989; **2**: S456–9.

50 Fahy JV, Kim KW, Liu J, Boushey HA. Prominent neutrophilic inflammation in sputum from subjects with asthma exacerbation. *J. Allergy Clin. Immunol.* 1995; **95**: 843–52.

51 Carroll N, Carello S, Cooke C, James A. Airway structure and inflammatory cells in fatal attacks of asthma. *Eur. Respir. J.* 1996; **9**: 709–15.

52 Debs RJ, Fuchs HJ, Philip R *et al.* Lung-specific delivery of cytokines induces sustained pulmonary and systemic immunomodulation in rats. *J. Immunol.* 1988; **140**: 3482–8.

53 Ulich TR, Watson LR, Yin S *et al.* The intratracheal administration of endotoxin and cytokines. *Am. J. Path.* 1991; **138**: 1485–96.

54 Michel O, Ginanni R, Le Bon B, Content J, Duchateau J, Sergysels R. Inflammatory response to acute inhalation of endotoxin in asthmatic patients. *Am. Rev. Respir. Dis.* 1992; **146**: 352–7.

55 Hallsworth MP, Soh CP, Lane SJ, Arm JP, Lee TH. Selective enhancement of GM-CSF, TNF alpha, IL-1 beta and IL-8 production by monocytes and macrophages of asthmatic subjects. *Eur. Respir. J.* 1994; **7**: 1096–102.

56 Cembrzynska-Nowak M, Szklarz E, Inglot AD, Teodorczyk-Injeyan JA. Elevated release of tumor necrosis factor-alpha and interferon-gamma by bronchoalveolar leukocytes from patients with bronchial asthma. *Am. Rev. Respir. Dis.* 1993; **147**: 291–5.

57 Gosset P, Tsicopulos A, Wallaert B, Joseph M, Capron A, Tonnel AB. Tumor necrosis factor alpha and interleukin-6 production by human mononuclear phagocytes from allergic asthmatics after IgE-dependent stimulation. *Am. J. Respir. Dis.* 1992; **146**: 768–74.

58 Ying S, Robinson DS, Varney V *et al.* TNF alpha mRNA expression in allergic inflammation. *Clin. Exp. Allergy* 1991; **21**: 745–50.

59 Lummus ZL, Alam R, Bernstein JA, Bernstein DI. Diisocyanate antigen-enhanced production of chemoattractant protein-1, IL-8, and tumour necrosis factor alpha by peripheral mononuclear cells of workers with occupational asthma. *J. Allergy Clin. Immunol.* 1998; **102**: 265–74.

60 Siracusa A, Vecchiarelli A, Brugnami G, Marabini A, Felicioni D, Severini C. Changes in interleukin-1 and tumor necrosis factor production by peripheral blood monocytes after specific bronchoprovocation test in occupational asthma. *Am. Rev. Respir. Dis.* 1992; **146**: 408–12.

61 Maestrelli P, di Stefano A, Occari A *et al.* Cytokines in the airway mucosa of subjects with asthma induced by toluene diisocyanate. *Am. J. Respir. Crit. Care Med.* 1995; **151**: 607–12.

62 Keatings VM, O'Connor BJ, Wright LG, Huston DP, Corrigan CJ, Barnes PJ. Late response to allergen is associated with increased concentrations of tumor necrosis factor alpha and IL-5 in induced sputum. *J. Allergy Clin. Immunol.* 1997; **99**: 693–8.

63 Pellegrino M, Minevini B, Musto P, Matera R, Greco A, Checchia de Ambrosio C. Tumor necrosis factor alpha and interleukin-1 beta. Two possible mediators of allergic inflammation. *Minerva Pediatrica* 1996; **48**: 309–12.

64 Koizumi A, Hashimoto S, Kobayashi T, Imai K, Yachi A, Horie T. Elevation of serum soluble vascular cell adhesion molecule-1 (sVCAM-1) levels in bronchial asthma. *Clin. Exp. Immunol.* 1995; **101**: 468–73.

65 Kobayashi T, Hashimoto S, Imai K, Amemiya E, Yamaguchi M, Yachi A, Horie T. Elevation of serum soluble intercellular adhesion molecule-1 (sICAM-1) and sE-selectin levels in bronchial asthma. *Clin. Exp. Immunol.* 1994; **96**: 110–15.

66 Bradding P, Roberts JA, Britten KM. *et al.* Interleukin-4-5, and -6 and tumor necrosis factor alpha in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am. J. Respir. Crit. Care Med.* 1994; **10**: 471–80.

67 Subratty AH, Hooloman NK. Role of circulating inflammatory cytokines in patients during an acute attack of bronchial asthma. *Indian J. Chest Dis. Allied Sci.* 1998; **40**: 17–21.

68 Sumitomo M, Suda S, Shindo K *et al.* Serum levels of tumor necrosis factor alpha and soluble tumor necrosis factor receptor I in asthmatic patients with chronic respiratory infection. *Arerugi-Jap. J. Allergology* 1997; **46**: 1136–47.

69 Nocker RE, van der Zee JS, Weller FR, van Overfeld FJ, Jansen HM, Out TA. Segmental allergen challenge induces plasma protein leakage into the airways of asthmatic subjects at 4 hours but not at 5 minutes after challenge. *J. Lab. Clin. Med.* 1999; **134**: 74–82.

70 Gelder CM, Thomas PS, Yates DH, Morrison JFJ, Barnes PJ. Cytokine expression in normal, atopic, and asthmatic subjects using the combination of sputum induction and the polymerase chain reaction. *Thorax* 1995; **50**: 1033–7.

71 Bodley KJ, Semper AE, Redington AE *et al.* Cytokine profiles of BAL T cells and T-cell clones obtained from human asthmatic airways after local allergen. *Allergy* 1999; **54**: 1083–93.
72 Divjak M, Glare EM, Bailey MJ, Walter EH. Tumour necrosis factor is down-regulated in asthma and upregulated by inhaled corticosteroid: comparative analysis by in situ hybridisation, immunocytochemistry and competitive RT-PCR in airway samples (Abstract). *Respirology* 2000; 5 (Suppl.): A3.
73 Robbins RA, Barnes PJ, Springall DR *et al.* Expression of inducible nitric oxide in human lung epithelial cells. *Biochem. Biophys. Res. Commun.* 1994; **203**: 209–18.
74 Kwon S, George SC. Synergistic cytokine-induced nitric oxide production in human alveolar epithelial cells. *Nitric Oxide* 1999; 3: 348–57.
75 Hamid Q, Springall DR, Riveros-Moreno V *et al.* Induction of nitric oxide synthase in asthma. *Lancet* 1993; **342**: 1510–3.
76 Yates DH, Kharitonov SA, Robbins RA, Thomas PS, Barnes PJ. Effect of a nitric oxide synthase inhibitor and a glucocorticoid on exhaled nitric oxide. *Am. J. Respir. Crit. Care Med.* 1995; **152**: 892–6.
77 Elliott MJ, Maini RN, Feldmann M *et al.* Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor α (cA2) versus placebo in rheumatoid arthritis. *Lancet* 1994; **344**: 1105–10.
78 Bigda J, Holtmann H. TNF receptors-how they function and interact. *Arch. Immunol. Ther. Exp.* 1997; **45**: 263–70.
79 Yuan J. Transducing signals of life and death. *Curr. Opin. Cell Biol.* 1997; **9**: 247–51.
80 Grell M, Zimmermann G, Gottfried E *et al.* Induction of cell death by tumour necrosis factor (TNF) receptor 2, CD40 and CD30: a role for TNF-R1 activation by endogenous membrane-anchored TNF. *EMBO J.* 1999; **18**: 3034–43.
81 Ismaair MG, Ries C, Lottspeich F, Zang C, Kolb HJ, Petrides PE. Autocrine regulation of matrix metalloproteinase-9 gene expression and secretion by tumor necrosis factor alpha (TNFα) in NB4 leukemic cells: specific involvement of TNF receptor type 1. *Leukemia* 1998; **12**: 1136–43.
82 Gaede KI, Fitschen J, Ernst M, Martinet N, Schlaak M, Muller-Quernheim J. Expression of tumour necrosis factor receptors (CD120a and CD120b) on bronchoalveolar cells. *Cytokine* 1999; **11**: 611–16.
83 Boussaud V, Soler P, Moreau J, Goodwin RG, Hance AJ. Expression of three members of the TNF-R family of receptors (4–1BB, lymphotoxin-beta receptor, and Fas) in the human lung. *Eur. Respir. J.* 1998; **12**: 926–31.
84 Liebmann PM, Reibnegger G, Lehofer M *et al.* Circadian rhythm of the soluble p75 tumor necrosis factor (sTNF-R75) receptor in humans—a possible explanation for the circadian kinetics of TNF-alpha effects. *Int. Immunol.* 1998; **10**: 1393–6.
85 Louis E, Franchimont D, Piron A *et al.* Tumour necrosis factor (TNF) gene polymorphism influences TNF alpha production in lipopolysaccharide (LPS) -stimulated whole blood cell culture in healthy humans. *Clin. Exp. Immunol.* 1998; **113**: 401–6.
86 Abraham LJ, Kroeger KM. Impact of the –308 TNF promoter polymorphism on the transcriptional regulation of the TNF gene: relevance to disease. *J. Leukocyte Biol.* 1999; **66**: 562–6.
87 Knight JC, Kwiatkowski D. Inherited variability of tumor necrosis factor production and susceptibility to infectious disease. *Proc. Assoc. Am. Physicians* 1999; **111**: 290–8.
88 Moffat MF, Cookson WO. Linkage and candidate gene studies in asthma. *Am. J. Respir. Crit. Care Med.* 1997; **156** (Suppl.): S110–2.
89 Moffat MF, Cookson WO. Tumour necrosis factor haplotypes and asthma. *Human Mol. Genet.* 1997; **6**: 551–4.
90 Moffat MF, James A, Ryan G, Musk AW, Cookson WO. Extended tumour necrosis factor/HLA-DR haplotypes and asthma in an Australian population sample. *Thorax* 1999; **54**: 757–61.
91 Albuquerque RV, Hayden CM, Palmer LJ *et al.* Association of polymorphism within the tumour necrosis factor genes and childhood asthma. *Clin. Exp. Allergy* 1998; **28**: 578–84.
92 Li Kam Wa TC, Mansur AH, Britton J *et al.* Association between –308 tumour necrosis factor promoter polymorphism and bronchial hyper-reactivity in asthma. *Clin. Exp. Allergy* 1999; **29**: 1204–8.
93 Changani T, Pare PD, Zhu S *et al.* Prevalence of tumor necrosis factor alpha and angiotensin converting enzyme polymorphisms in mild/moderate and fatal/near fatal asthma. *Am. J. Respir. Crit. Care Med.* 1999; **160**: 278–82.
94 Zhu S, Yeung M, Ward H *et al.* Prevalence for tumour necrosis factor alpha –308 promoter polymorphism in atopic and asthmatic subjects (Abstract). *Am. J. Respir. Crit. Care Med.* 1998; **157** (Suppl.): A773.
95 Kroeger KM, Steer JH, Joyce DA, Abraham LJ. Effects of stimulus and cell type on the expression –308 tumour necrosis factor promoter polymorphism. *Cytokine* 2000; **12**: 110–19.
96 Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc. Natl Acad. Sci. USA* 1997; **94**: 3195–9.
97 Uglialoro AM, Turbay D, Pesavento PA *et al.* Identification of three new single nucleotide polymorphisms in the human tumor necrosis factor alpha gene promoter. *Tissue Antigens* 1998; **52**: 359–67.
98 Armour A, Slocombe PM, Webster A *et al.* TNF-alpha converting enzyme (TACE) is inhibited by TIMP-3. *FEBS Lett.* 1998; **435**: 39–44.
99 Peschon JJ, Slack JL, Reddy P *et al.* An essential role for ectodomain shedding in mammalian development. *Science* 1996; **271**: 1281–4.
100 Mullberg J, Althoff K, Jostock T, Rose-John S. The importance of shedding of membrane proteins for cytokine biology. *Eur. Cytokine Network* 2000; **11**: 27–38.
101 Gearing AJH, Beckett P, Christodoulou M *et al.* Processing of tumour necrosis factor α precursor by metalloproteinases. *Nature* 1994; **370**: 555–7.
102 Steer JH, Kroeger KM, Abraham LJ, Joyce DA. Glucocorticoids suppress tumor necrosis factor alpha expression by human monocytic THP-1 cells by suppressing transactivation through adjacent NF-kappa b and c-Jun-activating transcription factor-2 binding sites in the promoter. *J. Biol. Chem.* 2000; **275**: 18 432–40.
103 Eigler A, Sinha B, Hartmann G, Endres S. Taming TNF strategies to restrain this proinflammatory cytokine. *Immunol. Today* 1997; **18**: 487–92.
104 Enzian P, Bitter-Suermann S, Burdon D, Ernst M, Schlaak M, Zabel P. Difference in the anti-inflammatory effects of theophylline: important for the development of asthma therapy? *Allergy* 1998; **53**: 749–54.
105 Moriera AL, Sampaio EP, Zmuidzinas A, Frindt P, Smith KA, Kaplan G. Thalidomide exerts its inhibitory action by enhancing mRNA degradation. *J. Exp. Med.* 1993; **177**: 1675–80.
106 Tavares JL, Wangoo A, Dilworth P, Marshall B, Kotecha S, Shaw RJ. Thalidomide reduces tumor necrosis factor alpha production by human alveolar macrophages. *Respir. Med.* 1997; **91**: 31–9.
107 Oliver SJ, Cheng TP, Banquerigo ML, Brahn E. The effect of thalidomide and 2 analogs on collagen induced arthritis. *J. Rhematol.* 1998; **25**: 964–9.

108 Tracey KJ. Suppression of TNF and other proinflammatory cytokines by the tetravalent guanylhydrazone CNI-1493. In: Levin J, Pollack M, Yokochi T, Nakano M. (eds) *Endotoxin and Sepsis: Molecular Mechanisms of Pathogenesis, Host Resistance and Therapy.* New York: Wiley-Liss, 1998; 335–43.

109 Mohler KM, Sleath PR, Fitzner JN *et al.* Protection against a lethal dose of endotoxin by an inhibitor of tumour necrosis factor processing. *Nature* 1994; 370: 218–20.

110 Hattori K, Hirano T, Miyajima H *et al.* A metalloproteinase inhibitor prevents lethal acute graft-versus-host disease in mice. *Blood* 1997; 90: 542–8.

111 Renzetti LM, Paciorek PM, Tannu SA *et al.* Pharmacological evidence for tumor necrosis factor as a mediator of allergic inflammation in the airways. *J. Pharmacol. Exp. Ther.* 1996; 278: 847–53.

112 Fekade D, Knox K, Hussein K *et al.* Prevention of Jarisch-Herxheimer reactions by treatment with antibodies against tumor necrosis factor alpha. *N. Eng. J. Med.* 1996; 335: 311–15.